

The Embryonic Area and so-called "Primitive Knot" in the Early Monotreme Egg.

By

Professors J. T. Wilson, F.R.S., and J. P. Hill, F.R.S.

With Plate 3, and 1 Text-figure.

SINCE the appearance, in 1906, of our "Observations on the Development of *Ornithorhynchus*" (1) there have appeared for the most part only isolated comments upon the facts there set forth and on our interpretation of them. This is doubtless owing to the absence or the extreme rarity of such material as might serve as a reliable basis for criticism of our results.

Such commentary as has been forthcoming has mainly had reference to a feature on which we laid considerable stress, namely, the apparent co-existence of a primitive knot, which, in consequence of its general similarity to that of reptiles, we interpreted as an archenteric knot, with a quite independent primitive streak. Having arrived at this conclusion, we were forced to interpret later phenomena in its terms. This involved an identification of the obvious Hensen's knot of a subsequent stage (our "post-gastrular") with the earlier primitive knot. We therefore sought to explain how the latter structure might come to be included in the later embryonic area by a process of forward extension of that proliferative area which in the earlier phase extends from, and is traversed by, the primitive streak.

Our interpretation of the primitive knot of *Ornithorhynchus* has been challenged both by Dr. Assheton and Prof. Keibel, who have, independently of one another, suggested a different explanation.

In a reference to our paper in a footnote to his account of the mammalian germ-layers, Keibel (2) makes the following observation: "In their [Wilson and Hill's] opinion the primitive node, which marks the position of the blastopore, comes into relation with the primitive streak only secondarily; originally it lies outside the embryonic shield. Yet it seems to me questionable if the structure which the authors regard as the primitive node in early stages is the same structure which they so designate in later stages. I have wondered whether the primitive node of younger stages may not be the yolk-navel."

Substantially the same position was taken up by Assheton in the course of his criticism of Hubrecht's views on the early ontogenetic phenomena in mammals. In this contribution (3) Assheton has gone beyond mere suggestion of an alternative interpretation, and has endeavoured to establish it both by an independent critical examination of our own facts and findings and by further evidence of what he regards as similar occurrences in the blastoderm of Sauropsida. He has also endeavoured to bring the facts as interpreted by him into line with the Entherian condition as manifested, e. g. in the rabbit.

Still more recently, Assheton has returned to this subject in a paper on "Tropidonotus and the Archenteric Knot of Ornithorhynchus" (4), in which he exhibits a striking parallel in respect of a knot-structure between the reptilian and prototherian blastoderms. "If," he says, in this latest contribution, "my comparison is a correct one, the archenteric knot of Ornithorhynchus with its anterior and posterior lips of the blastopore, and its 'commencement of true archenteric invagination' may be dismissed, and another stumbling-block will be removed from the path of the student of mammalian embryology" (p. 634).

In view of these important criticisms of our previously expressed views, we feel it incumbent upon us to indicate our opinion of their validity. We should, indeed, have done so at an earlier date had it not been for the difficulties in

the way of collaboration at the Antipodes and the absence of any specific occasion for a further publication. Such an occasion has now, however, offered itself in the form of an opportunity of together investigating another egg of *Ornithorhynchus*, of which some account will be given further on in this paper.

We now desire to say at the outset that even prior to the appearance of Assheton's second paper (4) we had both, independently of one another, become convinced of the justice of the main contentions of Keibel and Assheton and of the general adequacy of their re-interpretation of the condition we recorded as existing in the *Ornithorhynchus* blastoderm. Our "primitive" or "archenteric" knot in the early egg of *Ornithorhynchus*, we are now prepared to regard as a yolk-knot or yolk-navel, as one might term it, a structure to be explained on the general lines suggested independently by Keibel and Assheton.

The interesting parallel which Assheton (4) has recently traced between the "primitive knot" of *Ornithorhynchus* and a similar structure in *Tropidonotus* seems to afford a further convincing proof of the homology he had previously established, and the question that remains for us with regard to the knot itself merely concerns the detailed interpretation of the various parts of the structure and the manner of its production.

Whilst it can never be wholly palatable to have to confess to an error of interpretation, it is no small mitigation in this case to recognise that the developmental processes described by us in *Ornithorhynchus* now assume a less complicated aspect. We feel, with Assheton, that "another stumbling block has been removed from the path of the student of mammalian embryology."

When we come to consider the modifications in our former work necessitated by the newer point of view, we are surprised to note how circumscribed the error really is, and how little it affects the major part of our investigation. It is true that the mistaken interpretation occupies a very prominent

position in our work. But this was due rather to its apparent intrinsic interest and importance than to any really fundamental significance. Its withdrawal does not after all seriously violate our general conception of monotreme development outside the area of the error itself.

It is, of course, our interpretation of the so-called gastrular stage and of its supposed relationship to the stages immediately preceding and succeeding that is practically alone affected. Withdraw that interpretation and our descriptive account of these other stages remains valid and open to a less extraordinary and in some sense easier and more natural explanation.

The modifications of our former publication (1) which are demanded by our change of opinion may best be summarised by indicating the necessary amendments in the several published summaries on pp. 59-61, 90-91, and 116-17, of that work.

On p. 60, (*e*), the "primitive streak-area" here referred to must be regarded as an embryonic area, whilst the "axial thickening of the mesoderm" can only be a so-called "head-process." In (*f*), the "primitive knot" here referred to must be interpreted in terms of Assheton's and Keibel's suggestion.

Otherwise this summary holds good.

On pp. 90-91, the only amendment required is the deletion of proposition (*d*).

On pp. 116-117, the propositions expressed under the letters (*a*) to (*h*) can no longer be maintained. The remainder of this summary, in our opinion, still holds good. The conception implied in the term archenteron may be open to discussion, but our employment of the term is not now—and was not formerly—dependent merely on the view now discarded.

We have again examined the sections of the blastoderm of our former specimen "Q" in the region of the axial thickening of the mesoderm in front of the anterior end of the primitive streak. We now agree with Assheton that the embryonic area of our Text-fig. 7 expands into that of our

Text-fig. 8, as corresponding areas do in other mammals and in birds; whilst, as above noted, we now regard the axially thickened forward extension of the mesoderm in front of the primitive streak as a "head-process," and therefore as being directly related, as an early phase, to the long "archenteric" process of Text-fig. 8. Figs. 14-16 on Pl. 5 of our previous memoir represent cross-sections through the axial mesoderm in question. In our Text-fig. 7 the anterior limit, represented as that of the primitive streak, was fixed by, and actually indicates, the separation of the ectoderm from the mesodermal cell-thickening beneath. It thus really corresponds to the anterior limit of Hensen's knot, and any axial thickening of mesoderm in front of this must thus be "head-process." In our specimen "Q," such an axial thickening can be traced forwards in the sectional series through 42 sections in front of Hensen's knot. Thus the "head-process" should be plotted in, in Text-fig. 7, as extending 2.7 mm. (on the paper) in front of what is shown there as primitive streak, but whose anterior end actually represents Hensen's knot. It is to be noted, however, that neither the lateral nor the anterior limits of this head-process are sharp, but merge gradually into the thinner mesodermal sheet.

The absence from our collection of material of any stage which we could look upon as the immediate forerunner of our "gastrular" stage has been throughout a matter for regret. The specimen "a" of our paper was the only one at our disposal which at all approximated towards the gastrular. As appears from our paper, the examination of this egg showed completion of the bilaminar blastoderm, i. e. complete establishment of a bilaminar blastodermic vesicle in the mammalian sense, and one area of proliferative activity over the white yolk pole. This area showed a thickish cell-plate (our Pl. 2, text-fig. 5), forming a patch of about .5 mm. in its greatest diameter, which we took to be "the initial stage in the formation of the primitive knot." This opinion can no longer be maintained, and we are compelled to regard this area as simply the embryonic region of the blastoderm in an

early phase of its differentiation. The "accumulation of cells of irregular shapes and sizes, which appear to be actively proliferating," and which we formerly noted as existent "beneath the surface layer of the embryonic patch," and in proliferative continuity with the same, may very well represent the earliest product of that proliferative activity which gives origin to the primitive streak.

The strangest feature of the specimen as now interpreted is the entire absence of any trace of a yolk-navel or any equivalent structure. We have re-examined our material most carefully, and have found no trace whatever of any area which we could interpret as the site of coalescence of the margins of the blastoderm. In particular we can positively state that there is no such trace over the lower hemisphere of the egg. In the examination of a relatively large spheroidal structure, which necessarily has to be divided up for examination, it is impossible to be absolutely certain that nowhere near the lines of division could there have been some such trace. But at least none has been discoverable after the closest search; and we are, therefore, no nearer the solution of the problem of the yolk-knot after than before the examination of specimen "a."

The opportunity of examining another egg intermediate between our former specimens "a" and "Q" was, therefore, a very welcome one.

This egg, which appears in our list under the letters GW., was placed at our disposal by Prof. Gregg Wilson of Belfast, whose generous courtesy we desire here to acknowledge.

The egg was obtained at Gayndah, Queensland, in 1898, and was fixed in corrosive sublimate. As received by us it was somewhat collapsed and showed a rupture on one side. In this condition it measured 8.5×7.5 mm.

After cutting through the shell from the ruptured area, the blastocyst was separated in a more or less shrunken and collapsed state, and in this condition occupied a space of about 5.5×4 mm. It contained disseminated yolk material as well

as a more coherent yolk-mass, ovoidal and flattened in shape and about 4×2 mm. in diameter.

On examination in alcohol, an embryonic area traversed by a primitive streak was recognisable. It exhibited a primitive groove over about the anterior half of its extent. The length of the streak—neglecting the curvature of the blastoderm—was approximately 2.75 mm. and its breadth 0.3 to 0.4 mm. Throughout the greater part of its extent the primitive streak region showed as a longitudinal prominence on the surface of the somewhat shrunken blastoderm. This prominence was sharply accentuated at the anterior end of the streak, which here appeared to terminate in a knob-like thickening, representing a definite "Hensen's knot." The latter was readily recognisable by transmitted as well as by reflected light. Under transmitted light it appeared as a more opaque circular patch at the anterior extremity of the streak. It appeared as if definitely limited in front and no trace of a "head process" was perceptible on examination *in toto*. About 0.5 mm. anterior to Hensen's knot, a small local accumulation of yolk-spheres, about 0.5 mm. in diameter, was adherent to the deep surface of the blastoderm. Subsequent examination of the sections has not led us to attach any special significance to this patch.

After removal of the lower (antembryonic) polar area of the blastodermic vesicle, we sought very carefully for a yolk-knot or some equivalent appearance, but entirely without success. And we may at once state that the subsequent examination of the sections equally failed to afford any evidence of the existence of such a structure. As in the case of our former specimen "*a*," we are quite satisfied that no differentiated area of the kind was present over the lower polar area. For the same reasons as in the case of "*a*," there must remain a shade of uncertainty as regards the immediate vicinity of the lines of division of the blastoderm. At all events, we could detect nothing which even remotely suggested a yolk-knot, or indeed any differentiation other than the embryonic area itself. It is certainly most remarkable

that this should be the case when it is remembered in all four specimens of the only slightly later stage which we termed "gastrular" a relatively conspicuous yolk-knot (our "primitive knot") was found.

After preliminary infiltration in a 0.25 % solution of cedar oil-photoxylin, the upper hemisphere of the blastocyst was divided transversely into anterior and posterior segments and both portions as well as the lower hemisphere were embedded in paraffin and cut into serial sections of 8 μ . The total antero-posterior extent of the two portions of the upper hemisphere was represented by about 798 sections or just under 6.5 mm.

Examination of the sectional series showed that the primitive streak together with Hensen's knot extended through about 416 sections, or practically 3.3 mm. The anterior limit of Hensen's knot was easily defined by the abrupt cessation of its prominent convexity. But contrary to the suggestion of the surface examination an axial thickening of mesoderm extended forwards from the knot, forming a "head-process" of quite similar character to that we now recognise as existent in specimen "Q" of our former paper (cf. supra, p. 19). As in the latter case, so also here, this axial thickening of mesoderm is continuous, both bilaterally and in a forward direction, with the thinning out mesodermal sheet. As a "head-process" or recognisable axial thickening, it may be traced forwards for just one-third of a millimetre in front of the plane of its continuity with Hensen's knot.

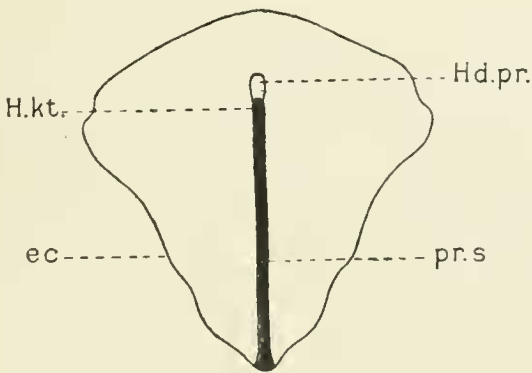
It is impossible in this specimen to determine definitely the hinder limit of Hensen's knot. As we formerly showed, such a limit does, at least at a later period, become distinguishable, probably as a result of inequalities in the growth rates of the different regions of the axial differentiation. In the present instance, a markedly convex prominence of the axial region of the embryonic area continues backwards from Hensen's knot, and the latter appears to merge gradually in the primitive streak tissue. It may be noted, however, that

examination of the blastoderm by transmitted light did indicate a posterior mass-limitation of Hensen's knot.

The sections show the entodermal sheet as everywhere complete. It continues across the embryonic region, underlying both the primitive streak and Hensen's knot. It is yolk-laden throughout, although, axially, where it underlies the latter structures, its cells appear as if more delicate in texture, suggesting a more active yolk-sphere digestion.

The yolk-entoderm of the general interior of the vesicle is of a character quite similar to that formerly described in

TEXT-FIG. 1.



Scheme of embryonic region of *Ornithorhynchus* Egg, GW, plotted to scale from the serial transverse sections. ($\times 6.25$)
ec. Outer limit of area of thick ectoderm. *H. kt.* Region of Hensen's knot. *Hd.-pr.* "Head-process." *pr. s.* Primitive streak.

nearly related stages. For purposes of comparison with the Text-figs. 7 and 8 of our previous paper, we have plotted the embryonic region of this specimen at the same magnification as that formerly employed (Text-fig. 1).

The embryonic area here shown is that definable by a thickening of the ectoderm, but here, as in the case of the other specimens, there is no abrupt line of demarcation, since the transition to the uniform thin ectoderm of the rest of the vesicle is a gradual one. The definite periphery shown must be accepted with this qualification. We have not thought it necessary to plot the limits of extension of the mesodermal

sheet. As might be expected, the extent of this sheet is considerably less than in our specimen "Q" (Text-fig. 7 of our previous paper). It is also distinctly weaker than in the latter case.

We have, however, plotted in, in front of the anterior limit of Hensen's knot, the extent of the axial mesodermal thickening or "head-process." Our former Text-fig. 7 (of specimen "Q") ought now to be amended, as we have already stated, by the insertion of a similar outline of the extent of the corresponding axial mesodermal thickening or "head-process," which we illustrated in the sectional Pl. 5, figs. 14-16, of our paper. If Text-fig. 7 were thus amplified, the "head-process" would appear as a projection in front of the anterior end of the primitive streak (really, here, Hensen's knot), extending for a distance in the figure of 2.7 mm. The breadth of the extension thus plotted would be about 4 mm. It is to be noted, however, that neither the anterior nor the lateral limits of the "head-process" so represented are sharply defined, but merge gradually in the thinner mesodermal sheet as seen, e. g. in our former Pl. 5, fig. 15.

LIST OF REFERENCES.

1. Wilson, J. T., and J. P. Hill.—"Observations on the Development of *Ornithorhynchus*," *Phil. Trans., Series B*, vol. 199, 1907.
2. Keibel, F., and F. P. Mall.—"Manual of Human Embryology," Lippincott Co., 1910, vol. i, pp. 46-47, footnote.
3. Assheton, R.—"Professor Hubrecht's paper on the Early Ontogenetic Phenomena in Mammals," *Quart. Journ. Micr. Sci.*, vol. 54, 1909-10.
4. ———. "Tropidonotus and the 'Archenteric Knot' of *Ornithorhynchus*," *Quart. Journ. Micr. Sci.*, vol. 54, 1909-10.

EXPLANATION OF PLATE 3,

Illustrating paper of Professors J. T. Wilson, F.R.S., and
J. P. Hill, F.R.S., on "The Embryonic Area and so-called
'Primitive Knot' in the Early Monotreme Egg."

Photomicrograph of embryonic area of Ornithorhynchus egg.
(G. W.). $\times 25$ diam.